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# PATENT SPECIFICATION

(11) 1206

1206233

## NO DRAWINGS

- (21) Application No. 2324/68 (22) Filed 16 Jan. 1968  
 (31) Convention Application No. 610 465 (32) Filed 20 Jan. 1967 in  
 (33) United States of America (US)  
 (45) Complete Specification published 23 Sept. 1970  
 (51) International Classification C 07 c 103/14  
 (52) Index at acceptance

C2C 1Q11J 1Q2 1Q6B1 1Q8A 1Q9A 1Q9C 1Q9D1 1Q9D2  
 1Q9L 20Y 220 226 227 22Y 29X 29Y 30Y 313 31Y  
 321 32Y 338 340 342 34Y 360 362 364 365 366 367  
 368 36Y 373 37Y 396 3A8A4 3A8B1 3A8C7 3A8J  
 491 581 591 620 627 628 62X 650 658 65X 70Y 738  
 757 799 KB KN LR RA RC RL

A2B 21  
 C6E 6D



## (54) ASPARTIC ACID DIPEPTIDE ALKYL ESTERS

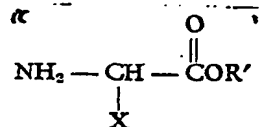
PATENTS ACT 1949

SPECIFICATION NO. 1,206,233

The following corrections were allowed under Section 76 on 19 March 1971

Page 1, line 16, after the formula insert '(I)'

Page 2, line 92, delete formula insert



Page 3, line 74, for '+ 4°C' read '+ 4°'

Page 3, line 96, for 'oxycarbonyl' read 'N-benzyloxycarbonyl'

Page 4, line 38, page 5, line 22, page 6, lines 4 and 5, for benzylcarbon read 'benzyloxycarbonyl'

Page 6, line 16, for 'hydrochlorice' read 'hydrochloric'

Attention is also directed to the following printers errors:-

Page 2, line 59, for 'a - n' read 'a - p'

Page 3, line 55, for 'tyrosine' read 'L-tyrosine'

Page 3, line 57, for '5°C' read '5°'

Page 3, line 72, after '235°' insert 'with'

Page 4, lines 30 and 31, for 'methyltyrosine' read 'ethyltyrosine'

Page 5, line 41, for 'ethyylcysteine' read 'ethylcysteine'

Page 6, line 118, for 'ethy' read 'ethyl'

Page 8, line 48, for 'contact' read 'contacting'

THE PATENT OFFICE  
 22 April 1971

[Price -

SEE CORRECTION SLIP ATTACHED

# PATENT SPECIFICATION

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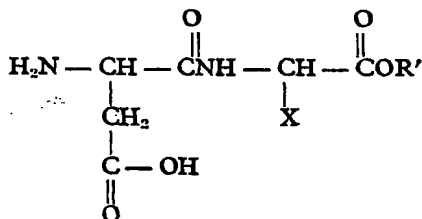
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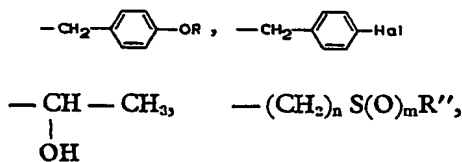
## (54) ASPARTIC ACID DIPEPTIDE ALKYL ESTERS

(71) We, G. D. SEARLE & Co., a corporation organised and existing under the laws of the State of Delaware, United States of America of Post Office Box 5110, Chicago, Illinois 60680, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to aspartic acid dipeptide alkyl esters and to the processes for their preparation. More particularly, the compounds prepared by this invention have the following general formula



wherein X is a radical of the formula



20

or —R''';

Hal is a fluorine, chlorine, bromine, or iodine atom; m is 0 or 2; n is 1 or 2; R is

hydrogen or a straight or branched chain alkyl radical containing from 1 to 7 carbon atoms; and R', R'', and R''' are each a straight or branched chain alkyl radical containing from 1 to 7 carbon atoms; with the provision that when R''' contains 1 or 2 carbon atoms, R' must contain more than 2 carbon atoms.

The dipeptide derivatives of the present invention share the surprising and completely unexpected property of possessing a sweet taste. This property is useful as a result of the ability of those derivatives to impart their sweetness to a variety of food products. Examples of such food products are fruits, vegetables, juices, meat products such as ham or bacon, sweetened milk products, egg products, salad dressings, ice creams and sherbets, gelatins, icings, syrups, cake mixes and beverages such as carbonated soft drinks and wines.

An example of a typical sweetened orange soda is shown by the following preparation:

A stock supply of bottler's syrup was prepared by mixing 5.5 ml. of a 50% aqueous citric acid solution with 150 ml. of water, 2 g. of L - aspartyl - L - tyrosine methyl ester were dissolved in that solution, and 7.02 ml. of the orange flavour base manufactured by the A. E. Illes Company, Dallas, Texas, labelled FO—78 and 2.7 g. of sodium benzoate were added and the mixture diluted to 200 ml. with water. One oz. samples of that bottler's syrup were transferred to 6 oz. bottles and 110 ml. of cold tap water added to each bottle. To each bottle 42 ml. of cold charged bottling water (5 volumes carbon dioxide) was then added to achieve carbonation. Each bottle was capped and the contents mixed.

Comparison of the latter samples with orange soda containing a quantity of sucrose

SEE CORRECTION SLIP ATTACHED

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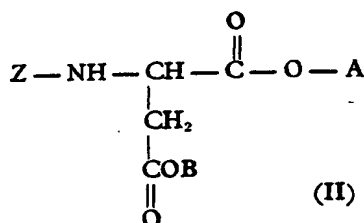
50 times that of the named dipeptide derivative revealed no detectable difference in sweetness.

5 The dipeptide sweetening agents are water soluble, stable substances which can be used in a variety of physical forms, for example as powders, tablets or syrups. Liquid or solid carriers such as water, glycerol, starch, sorbitol, salt, citric acid and other suitable non-toxic substances can be used also.

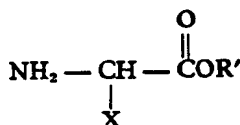
10 It has been determined that the property of sweetness is affected by the stereochemistry of the individual amino acid units comprising the dipeptide structure. The L-L isomers, for example L - aspartyl - L - tyrosine methyl ester, are especially sweet. It is thus apparent that mixtures containing the L-L isomer, i.e. DL-DL, L-DL or DL-L share that property also.

20 The sweetening agents of the present invention are particularly useful to diabetics as substitutes for sugar. They are, moreover, lacking in the unpleasant after taste exhibited by such synthetic sweeteners as saccharin and cyclamate. The absence of toxic properties results from their derivation from natural sources, i.e. the naturally occurring amino acids utilised by the animal body in the manufacture of essential proteins.

30 The novel compounds of formula (I) are conveniently produced by contacting an L-aspartic acid derivative of the formula



35 wherein Z is an amino protecting group such as the function trityl, *p*-nitrobenzyloxycarbonyl, *p*-bromobenzyloxycarbonyl or benzyloxycarbonyl, B is a benzyl radical which forms a protective ester moiety and A is a *p*-nitrophenyl or alkoxycarbonyl radical forming a labile oxygenated function, with an appropriate amino acid ester of the general formula



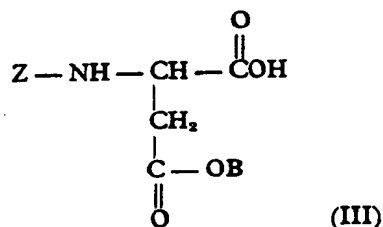
45 The protecting groups are then removed by suitable means from the aspartic acid portion of the resulting intermediate product. When the amino group is protected by a benzyloxycarbonyl function and the  $\beta$ -carboxy group by a benzyl ester moiety, catalytic hydrogenolysis is a preferred method for effecting removal.

Time and temperature are not critical factors but, as apparent to one skilled in the art, are dependent upon the particular solvent employed. Pressure is not critical, the reactions are, however, conveniently carried out at atmospheric or slightly elevated pressure.

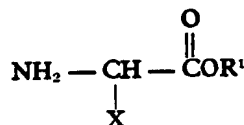
55 These processes are specifically illustrated by the reaction of N - benzyloxycarbonyl - L-aspartic acid  $\alpha$  - *n* - nitrophenyl,  $\beta$ -benzyl diester (described by S. Guttmann, *Helv. Chim. Acta*, 44 721 (1961) with L-tyrosine methyl ester to yield  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl - L - tyrosine methyl ester, which is hydrogenolyzed in aqueous acetic acid, using palladium black catalyst, to afford L - aspartyl - L - tyrosine methyl ester.

70 The dipeptides characterised by a sulphone group are obtained by reaction of the protected aspartic acid derivative with the appropriate amino acid ester containing the sulphone group. The latter substances are conveniently produced by esterification of the corresponding amino acid sulphones. For example, L-methionine sulphone is converted to its methyl ester hydrochloride by reaction with a solution of thionyl chloride in methanol. The free ester is allowed to react with the aforementioned N - benzyloxycarbonyl - L-aspartic acid  $\alpha$  - *p* - nitrophenyl  $\beta$ -benzyl diester to yield  $\beta$ -benzyl N-benzyloxycarbonyl - L - aspartyl - L - methionine methyl ester sulphone, which is hydrogenolyzed with palladium to afford L - aspartyl - L - methionine methyl ester sulphone.

85 Alternatively, the compounds of formula (I) can be prepared by contacting a compound of the general formula



90 wherein Z and B are as hereinabove defined, with an amino ester of the formula



95 in the presence of a suitable dehydrating agent and then removing the protecting groups by suitable means. Especially preferred dehydrating agents include alkylaminoacetylenes and preferably dicyclohexylcarbodiimide. When the amino group is protected by a benzyloxycarbonyl function and the  $\beta$ -carboxy group

by a benzyl ester moiety, catalytic hydrogenolysis is a preferred method for effecting removal. Time and temperature are not critical factors, but as apparent to one skilled in the art, are dependent upon the particular solvent employed. Pressure is not critical; the reactions are, however, conveniently carried out at atmospheric or slightly elevated pressure. Thus, when N - benzyloxycarbonyl - L - aspartic acid  $\beta$ -benzyl ester is reacted with L-tyrosine methyl ester in the presence of dicyclohexylcarbodiimide,  $\beta$  - benzyl N-benzyloxycarbonyl - L - aspartyl - L - tyrosine methyl ester is afforded which is then hydrogenolyzed in aqueous acetic acid, using palladium black catalyst, to afford L - aspartyl - L - tyrosine methyl ester.

Alternatively, the compounds of formula (I) can be prepared by reacting L-aspartic acid with phosgene to form the N-carboxyanhydride of L-aspartic acid and then reacting the latter substance with an appropriate amino ester. Time and temperature are not critical factors but are dependent upon the particular solvent employed. Thus when the N-carboxyanhydride of L-aspartic acid, formed by reacting L-aspartic acid with phosgene, is reacted with L-tyrosine methyl ester, L - aspartyl - L - tyrosine methyl ester is afforded.

The invention is further illustrated by the following examples. In these examples temperatures are given in degrees Centigrade ( $^{\circ}\text{C}$ ) and quantities of materials are expressed in parts by weight except where otherwise noted. The double melting points observed in the case of the dipeptide esters result from heat-catalysed cyclisation of those substances to afford the corresponding diketopiperazines.

#### EXAMPLE 1

A mixture containing 10.05 parts of N-benzyloxycarbonyl - L - aspartic acid  $\alpha$  - *p*-nitrophenyl,  $\beta$ -benzyl diester, L-tyrosine methyl ester and 45 parts of ethyl acetate was stored at about  $65^{\circ}$  for approximately 24 hours and then cooled and washed successively with 50% aqueous potassium carbonate, water, dilute hydrochloric acid and water. Drying of the solution over magnesium sulphate was followed by distillation of the solvent under reduced pressure and a gummy residue was obtained, which was purified by crystallisation from ether-ethyl acetate to afford  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl-tyrosine methyl ester, melting at 125 to  $127.5^{\circ}$  and exhibiting an optical rotation, in methanol, of  $-5^{\circ}\text{C}$ .

#### EXAMPLE 2

To a solution of 20 parts of  $\beta$  - benzyl - N-benzyloxycarbonyl - L - aspartyl - L - tyrosine methyl ester in 250 parts of 75% aqueous acetic acid there were added 2 parts of palladium black and the resulting mixture was

shaken with hydrogen at atmospheric pressure and room temperature until the uptake of hydrogen ceased. The catalyst was removed by filtration and the filtrate concentrated to dryness under reduced pressure. The resulting residue was crystallised from aqueous ethanol to afford L - aspartyl - L - tyrosine methyl ester, which exhibited a double melting point at 180 to  $185^{\circ}\text{C}$  and 230 to  $235^{\circ}$  decomposition. This compound displays an optical rotation, in water, of  $+4^{\circ}\text{C}$ .

#### EXAMPLE 3

3.4 Parts of N - benzyloxycarbonyl - L-aspartic acid  $\beta$ -benzyl ester were dissolved in 5 parts by volume tetrahydrofuran. 1 Part triethylamine was added and the solution cooled to  $-10^{\circ}$  with stirring; then 1 part ethyl chloroformate was added keeping the temperature below  $0^{\circ}$ . After about 10 minutes 2 parts L-tyrosine methyl ester in 5 parts by volume tetrahydrofuran were added to the solution keeping the temperature below  $0^{\circ}$ . The solution was allowed to stand approximately 16 hours at  $5^{\circ}$ . The triethylamine hydrochloride was removed by filtration and the filtrate stripped of solvent by distillation under reduced pressure to afford a gummy residue which was recrystallised from ether-ethyl acetate to afford  $\beta$ -benzyl N-benzyloxycarbonyl - L - aspartyl - L - tyrosine methyl ester, identical to the product of Example 1.

Using an equivalent amount of  $\beta$ -benzyloxycarbonyl - L - aspartyl - L - tyrosine methyl ester and following the procedure of Example 2, there was obtained L - aspartyl-L - tyrosine methyl ester identical to the product of Example 2.

#### EXAMPLE 4

The substitution of 4.62 parts of L-tyrosine ethyl ester in the procedure of Example 1 followed by recrystallisation of the crude product from ether-isopropyl acetate affords  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl-tyrosine ethyl ester, melting at 122 to  $124^{\circ}$  and exhibiting an optical rotation, in methanol, of  $-9^{\circ}$ .

When 5 parts of the above prepared  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl-L - tyrosine ethyl ester were substituted in the procedure of Example 2, there was produced L - aspartyl - L - tyrosine ethyl ester, exhibiting a melting point of 188 to  $190^{\circ}$  with effervescence and an optical rotation, in water, of  $+6^{\circ}$ .

#### EXAMPLE 5

To a solution of 5.73 parts of L - O-methyltyrosine methyl ester hydrochloride in 50 parts of water was added sufficient potassium bicarbonate to effect neutralisation, and the resulting aqueous mixture was extracted with ethyl acetate. The organic layer was separated, washed with water and dried over anhydrous magnesium sulphate.

When the latter organic solution was substituted for L-tyrosine methyl ester in the procedure of Example 1, there was produced  $\beta$ -benzyl N - benzylcarbonyl - L - aspartyl-L - O - methyltyrosine methyl ester, which, after recrystallisation from ether-ethyl acetate, melts at 114 to 115°C and exhibits an optical rotation, in methanol, of  $-4.5^\circ$ .

By substituting 5 parts of the above prepared  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl - L - O - methyltyrosine methyl ester in the procedure of Example 2 there was produced L - aspartyl - L - O - methyltyrosine methyl ester, which exhibits a double melting point at 138 to 140° and 185 to 235° with decomposition. This compound possesses an optical rotation of  $-0.5^\circ$ .

#### EXAMPLE 6

When 7.65 parts of L - O - ethyltyrosine methyl ester hydrochloride were substituted in the procedure of Example 5 there was produced  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl - L - O - ethyltyrosine methyl ester, melting at 115 to 116° and displaying an optical rotation, in methanol, of  $-6^\circ$ .

The hydrogenolysis of 5 parts of the above prepared  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl - L - O - ethyltyrosine methyl ester by the procedure described in Example 2 resulted in L - aspartyl - L - O - methyltyrosine methyl ester, exhibiting a melting point at 185 to 240° with decomposition. It displays an optical rotation, in methanol, of  $-1.5^\circ$ .

#### EXAMPLE 7

To a mixture containing 6 parts of L-methionine methyl ester hydrochloride, 15.3 parts of N - benzylcarbonyl - L - aspartic acid  $\alpha$  - p - nitrophenyl,  $\beta$ -benzyl diester and 38 parts of dimethylformamide were added with cooling and stirring, 3.3 parts of triethylamine. Stirring was continued for about 2 hours; the mixture stored at about 8° for approximately 16 hours and stirring continued at room temperature for from 3 to 4 hours. The mixture was then diluted with ethyl acetate and washed successively with dilute hydrochloric acid, dilute aqueous potassium carbonate and water. The aqueous washings were extracted with ethyl acetate and the organic extracts combined with the original organic solution. The combined solutions were filtered through sodium sulphate and the solvent allowed to evaporate at room temperature. The resulting residue was purified by recrystallisation from isopropyl alcohol followed by washing with ether and drying in air to yield  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl - L - methionine methyl ester, melting at 78 to 78.5° and exhibiting an optical rotation, in chloroform, of  $+26.5^\circ$ .

To a solution of 10 parts of the above prepared  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl - L - methionine methyl ester in 120

parts by volume of 75% aqueous acetic acid were added 3 parts of palladium black catalyst, and the resulting mixture shaken with hydrogen at atmospheric pressure and room temperature until the uptake of gas ceased. Separation of the catalyst by filtration followed by distillation of the solvent under reduced pressure afforded the residual crude product, which was purified first by trituration with ether, then by recrystallisation from methanol-ether to afford pure L - aspartyl-L - methionine methyl ester, which exhibited a triple melting point at 111°, 136.5 to 145° with decomposition and 200 to 213.5°. This compound displayed an optical rotation, in water, of  $-19.5^\circ$ .

#### EXAMPLE 8

To a mixture containing 5.1 parts of L-threonine methyl ester hydrochloride, 15.1 parts of N - benzyloxycarbonyl - L - aspartic acid  $\alpha$ -p-nitrophenyl,  $\beta$ -benzyl diester and 38 parts of dimethyl-formamide were added, with cooling, 3.3 parts of triethylamine, and the resulting reaction mixture was stirred for about 1 hour, then allowed to stand at room temperature for about 16 hours. At this point 0.5 part of imidazole was added and the reaction mixture stirred for about 1 hour and then diluted with ethyl acetate and washed successively with dilute hydrochloric acid, dilute aqueous potassium bicarbonate and dilute aqueous sodium sulphate. The organic solution was dried over anhydrous sodium sulphate and the solvent allowed to evaporate at room temperature, thus resulting in  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl - L - threonine methyl ester.

To a solution of 11 parts of the above prepared  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl - L - threonine methyl ester in 200 parts by volume of 75% aqueous acetic acid was added 1 part of palladium black catalyst and the mixture was shaken with hydrogen at atmospheric pressure and room temperature until the uptake of hydrogen was complete. The catalyst was removed by filtration and the filtrate concentrated to dryness under reduced pressure. The residue was dissolved in aqueous methanol and the crude product precipitated by the addition of ether. Purification of the precipitated crude product was effected by recrystallisation from aqueous isopropyl alcohol to yield pure L - aspartyl - L - threonine methyl ester, melting at 159 to 160.5° with decomposition. It displays an optical rotation, in water, of  $-6^\circ$ .

#### EXAMPLE 9

A mixture containing 7.45 parts of L - S-methylcysteine methyl ester hydrochloride, 20.1 parts of N - benzyloxycarbonyl - L - aspartic acid  $\alpha$ -p-nitrophenyl,  $\beta$ -benzyl diester and 47.5 parts of dimethylformamide was stirred, then cooled while 4.3 parts of

triethylamine were added dropwise. Stirring at room temperature was continued for about 1½ hours, at the end of which time the mixture was stored for about 20 hours. Approximately 0.5 part of imidazole was added and the mixture stirred for about 1 hour, and then diluted with ethyl acetate. The resulting organic solution was washed successively with dilute hydrochloric acid, dilute aqueous potassium carbonate and dilute aqueous sodium sulphate. The washed solution was dried over anhydrous sodium sulphate, then stripped of solvent by distillation under reduced pressure to afford the residual crude product which, after recrystallisation from ether, afforded pure β-benzyl N - benzyloxycarbonyl - L - aspartyl - L - S - methylcysteine methyl ester displaying a double melting point at 55 to 62° and 76 to 82°. This compound possesses an optical rotation, in chloroform, of +15.5°.

A mixture containing 7.3 parts of the above prepared β-benzyl N - benzyloxycarbonyl - L - aspartyl - L - S - methylcysteine methyl ester, 120 parts by volume of 75% aqueous acetic acid and 1.4 parts of palladium black catalyst was shaken with hydrogen at atmospheric pressure and at room temperature until the uptake of hydrogen ceased, at which time the catalyst was removed by filtration and the solvent distilled under reduced pressure. The resulting residual crude product was triturated with ether and then further purified by recrystallisation from methanol-isopropyl alcohol and washed with ether to yield pure L - aspartyl - L - S - methylcysteine methyl ester which exhibited a melting point at 146.5 to 149° with decomposition and displayed an optical rotation, in water, of -26°.

#### EXAMPLE 10

When an equivalent quantity of L - S - ethylcysteine methyl ester hydrochloride was substituted in the procedure of Example 9, there was produced β-benzyl N - benzyloxycarbonyl - L - aspartyl - L - S - ethylcysteine methyl ester.

The substitution of an equivalent quantity of β-benzyl N - benzyloxycarbonyl - L - aspartyl - L - S - ethylcysteine methyl ester in the procedure to remove blocking groups of Example 9 resulted in L - aspartyl - L - S - ethylcysteine methyl ester.

#### EXAMPLE 11

To 40 parts of cold methanol was added dropwise 4.9 parts of thionyl chloride, and 6 parts of L-methionine sulphone were added to the latter solution. The resulting reaction mixture was stirred at the reflux temperature for 2½ hours and then cooled and stored at room temperature for 16 hours. Decolourisation of the solution with activated carbon followed by dilution with ether resulted in precipitation of the product, which was col-

lected by filtration and washed with ether to yield L-methionine sulphone methyl ester hydrochloride, melting at 164 to 167° with decomposition and exhibiting an optical rotation, in water, of +25°.

#### EXAMPLE 12

A mixture of 4.65 parts of L-methionine sulphone methyl ester hydrochloride, 10.05 parts of N - benzyloxycarbonyl - L - aspartic acid α-p-nitrophenyl, β-benzyl diester and 23.8 parts of dimethylformamide was stirred while 2.15 parts of triethylamine were added dropwise. Stirring at room temperature was continued for about 1½ hours longer, following which time the mixture was stored at room temperature for about 16 hours. At the end of that time, ethyl acetate was added and the resulting organic solution washed successively with dilute hydrochloric acid, dilute aqueous potassium carbonate and dilute aqueous sodium acetate. The aqueous washings were extracted with ethyl acetate and the combined organic solutions dried over anhydrous sodium sulphate, then allowed to stand in order to permit the solvent to evaporate. The resulting residual crude product was purified by recrystallisation from isopropyl alcohol to afford pure β-benzyl N - benzyloxycarbonyl - L - aspartyl - L - methionine sulphone methyl ester, melting at 84 to 112°. This compound exhibited an optical rotation, in chloroform, of +30°.

To a solution of 5.35 parts of the above prepared β-benzyl N - benzyloxycarbonyl - L - aspartyl - L - methionine sulphone methyl ester in 150 parts by volume of 75% aqueous acetic acid was added 0.5 part of palladium black catalyst and the resulting mixture was shaken with hydrogen at room temperature and atmospheric pressure until cessation of hydrogen uptake. Removal of the catalyst by filtration and the solvent by distillation under reduced pressure yielded the crude product which was triturated with ether, then further purified by recrystallisation from methanol-isopropyl alcohol to yield L - aspartyl - L - methionine sulphone methyl ester having a melting point at 155 to 156° with decomposition and an optical rotation, in water, of -2°.

#### EXAMPLE 13

To 40 parts of methanol was added dropwise 5.74 parts of thionyl chloride, following which 4.24 parts of L - S - methyl - cysteine sulphone were added. The resulting reaction mixture was heated at the reflux temperature for 2 hours then cooled and decolourised with activated carbon. Dilution of the solution with ether yielded L - S - methylcysteine sulphone methyl ester hydrochloride, melting at 168 to 170° with decomposition and displaying an optical rotation, in water, of +6°.

## EXAMPLE 14

To a solution of 4.35 parts of L - S-methylcysteine sulphone methyl ester hydrochloride and 10.05 parts of N - benzyl-carbonyl - L - aspartic acid  $\alpha$ -*p*-nitrophenyl,  $\beta$ -benzyl diester in 23.8 parts of dimethyl-formamide were added, with stirring and cooling, 2.15 parts of triethylamine. Stirring at room temperature was continued for about 2 hours, following which time the mixture was stored for about 16 hours. Dilution with ethyl acetate followed by successive washing with dilute hydrochloric acid, dilute aqueous potassium carbonate and dilute aqueous sodium sulphate resulted in the organic solution. The dilute hydrochloric acid, dilute aqueous potassium carbonate and dilute aqueous sodium sulphate washings from the above were extracted with ethyl acetate. These ethyl acetate extracts were then combined with the organic solution obtained above. This was then dried over anhydrous sodium sulphate and stripped of solvent by distillation under reduced pressure. The resulting residue was crystallised from isopropyl alcohol, then washed with cyclohexane and pentane to yield  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl - L - S - methylcysteine sulphone methyl ester, melting at 96 to 98°. The compound exhibits an optical rotation, in chloroform, of +14°.

To a solution of 7.03 parts of the above prepared  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl - L - S - methylcysteine sulphone methyl ester in 150 parts by volume of 75% aqueous acetic acid was added 0.7 part of palladium black catalyst and the resulting reaction mixture was shaken with hydrogen at atmospheric pressure and room temperature until hydrogen was no longer absorbed. The catalyst was removed by filtration and the solvent distilled under reduced pressure to yield the crude residual product. Trituration with ether yielded a gummy solid, which was purified by crystallisation from aqueous isopropyl alcohol to yield L - aspartyl - L - S - methylcysteine sulphone methyl ester, melting at 135 to 136° with decomposition and displaying an optical rotation, in water, of -9°.

## EXAMPLE 15

Substitution of an equivalent quantity of DL-*p*-fluorophenylalanine in the procedure of Example 11 resulted in DL-*p*-fluorophenylalanine methyl ester hydrochloride melting at 176.5 to 178.5°.

## EXAMPLE 16

Substitution of an equivalent quantity of DL-*p*-fluorophenylalanine methyl ester hydrochloride in the procedure of Example 12 resulted in  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl - DL - *p* - fluorophenylalanine methyl ester.

Substitution of an equivalent quantity of the above prepared  $\beta$ -benzyl N - benzyloxy-

carbonyl - L - aspartyl - DL - *p* - fluorophenylalanine methyl ester in the procedure to remove blocking groups of Example 12 yielded L - aspartyl - DL - *p* - fluorophenylalanine methyl ester.

## EXAMPLE 17

Substitution of an equivalent quantity of DL - *p* - chlorophenylalanine in the procedure of Example 11 resulted in DL-*p*-chlorophenylalanine methyl ester hydrochloride.

## EXAMPLE 18

Substitution of an equivalent quantity of DL - *p* - chlorophenylalanine methyl ester hydrochloride in the procedure of Example 12 resulted in  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl - DL - *p* - chlorophenylalanine methyl ester.

Substitution of an equivalent quantity of the above prepared  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl - DL - *p* - chlorophenylalanine methyl ester in the procedure to remove blocking groups of Example 12 yielded L - aspartyl - DL - *p* - chlorophenylalanine methyl ester.

## EXAMPLE 19

By substitution of an appropriate quantity of DL - 2 - aminoheptanoic acid and otherwise proceeding according to the process of Example 11 there was obtained DL-2-aminoheptanoic acid methyl ester hydrochloride.

## EXAMPLE 20

Substitution of an appropriate quantity of DL-2-aminoheptanoic acid methyl ester hydrochloride in the procedure of Example 12 resulted in  $\beta$  - benzyl - N - benzyloxycarbonyl - L - aspartyl - DL - 2 - aminoheptanoic acid methyl ester.

By substituting an appropriate quantity of the above prepared  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl - DL - 2 - aminoheptanoic acid methyl ester and otherwise proceeding according to the process to remove blocking groups of Example 12 there was obtained L - aspartyl - DL - 2 - aminoheptanoic acid methyl ester.

## EXAMPLE 21

A suspension of 1.3 parts L-aspartic acid in anhydrous dioxane was stirred over phosgene at room temperature for approximately 30 minutes until all the reactant had dissolved. Then the solvent was removed by distillation under reduced pressure and the resulting crystalline substance purified by recrystallisation from ethyl acetate-petroleum ether to afford the N-carboxyanhydride of L-aspartic acid, i.e., L - 2,5 - oxazolidinedione - 4 - acetic acid.

1.6 parts of the above prepared L - 2,5-oxazolidinedione - 4 - acetic acid were dissolved in methylene chloride and cooled to -60°. To this solution were added 2 parts

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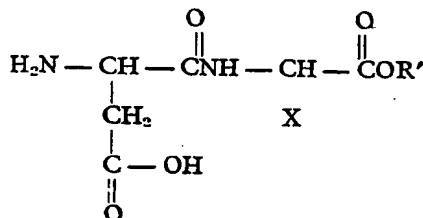
- 5 L-tyrosine methyl ester, 10 parts ethyl acetate and 1 part triethylamine. The resulting solution was allowed to stand to room temperature for approximately 24 hours. The solvent was then removed by distillation under reduced pressure and the resulting residue recrystallised from aqueous ethanol to which 0.6 part acetic acid had been added to yield a compound identical to the product of Example 2.

10 **EXAMPLE 22**

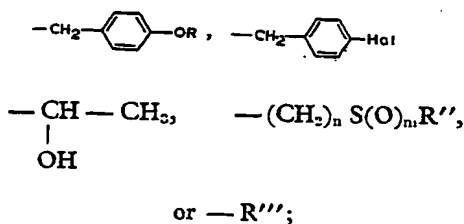
- 15 A mixture of 3.4 parts of N - benzyloxycarbonyl - L - aspartic acid  $\beta$ -benzyl ester, 1.95 parts L-tyrosine methyl ester, 2 parts dicyclohexylcarbodiimide and 10 parts dimethyl formamide was stirred with cooling for approximately 2 hours, then allowed to stand at room temperature for about 16 hours. The resulting mixture was filtered and the filtrate stripped of solvent by distillation under reduced pressure and the resulting residue recrystallised from ether-ethyl acetate to yield  $\beta$ -benzyl N - benzyloxycarbonyl - L-aspartyl - L - tyrosine methyl ester, identical to the product of Example 1.
- 25 Using an equivalent amount of the above prepared  $\beta$ -benzyl N - benzyloxycarbonyl-L - aspartyl - L - tyrosine methyl ester and following the procedure of Example 2 there was obtained L - aspartyl - L - tyrosine methyl ester identical with the product of Example 2.

WHAT WE CLAIM IS:—

1. A compound of the general formula



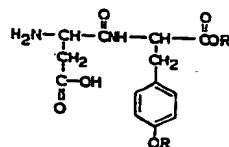
- 35 wherein X is a radical of the formula



- 40 Hal is a fluorine, chlorine, bromine, or iodine atom;  $m$  is 0 or 2;  $n$  is 1 or 2; R is hydrogen or a straight or branched chain alkyl radical containing from 1 to 7 carbon atoms; and R', R'', and R''' are each a straight or branched chain alkyl radical containing from 1 to 7 carbon atoms; with the provision that

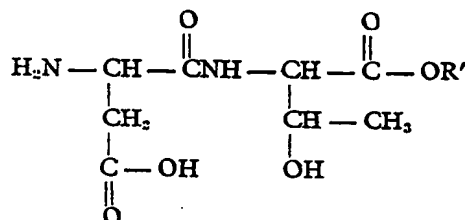
when R''' contains 1 or 2 carbon atoms, R' must contain more than 2 carbon atoms.

2. A compound of the general formula



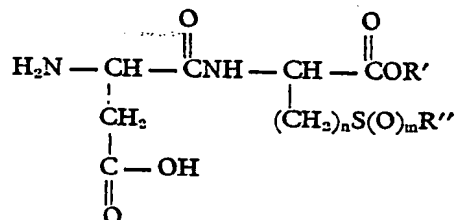
wherein R is hydrogen or a straight or branched chain alkyl radical containing from 1 to 7 carbon atoms, and R' is a straight or branched chain alkyl radical containing from 1 to 7 carbon atoms.

3. L - Aspartyl - L - tyrosine methyl ester. 55  
4. L - Aspartyl - L - tyrosine ethyl ester.  
5. L - Aspartyl - L - O - methyltyrosine methyl ester.  
6. L - Aspartyl - L - O - ethyltyrosine methyl ester. 60  
7. A compound of the general formula



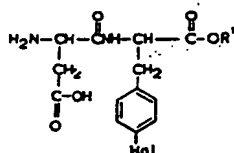
wherein R' is a straight or branched chain alkyl radical containing from 1 to 7 carbon atoms.

8. L - Aspartyl - L - threonine methyl ester. 65  
9. A compound of the general formula



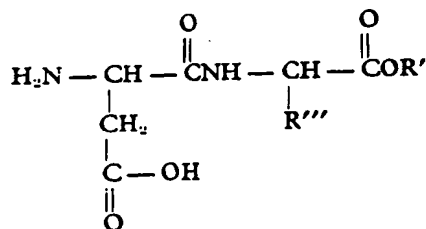
wherein  $m$  is 0 or 2,  $n$  is 1 or 2, and R' and R'' are each a straight or branched chain alkyl radical containing from 1 to 7 carbon atoms.

10. L - Aspartyl - L - methionine methyl ester. 75  
11. L - Aspartyl - L - S - methylcysteine methyl ester.  
12. L - Aspartyl - L - S - ethylcysteine methyl ester.  
13. L - Aspartyl - L - methionine sulphone methyl ester. 80  
14. L - Aspartyl - L - S - methylcysteine sulphone methyl ester.  
15. A compound of the general formula



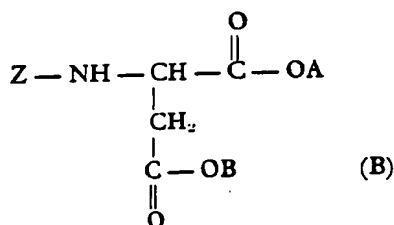
wherein Hal is a fluorine, chlorine, bromine or iodine atom, and R' is a straight or branched chain alkyl radical containing from

- 1 to 7 carbon atoms.  
 16. L - Aspartyl - DL - *p* - fluorophenyl-alanine methyl ester.  
 17. L - Aspartyl - DL - *p* - chlorophenyl-alanine methyl ester.  
 18. A compound of the general formula

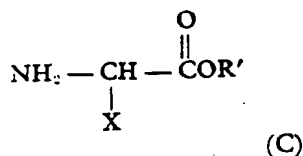


wherein R' and R''' are each a straight or branched chain alkyl radical containing from 1 to 7 carbon atoms, with the provision that when R''' contains 1 or 2 carbon atoms, R' must contain more than 2 carbon atoms.

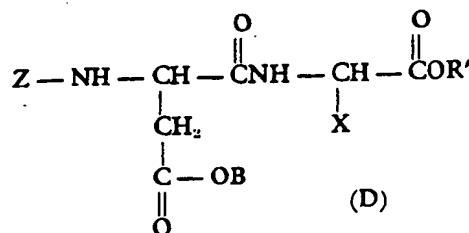
19. Compounds as claimed in claim 1 substantially as described with reference to the Examples 2 to 10, 12, 14, 16 and 18.  
 20. A process for the preparation of compounds as claimed in claim 1 which comprises contacting a compound of the general formula



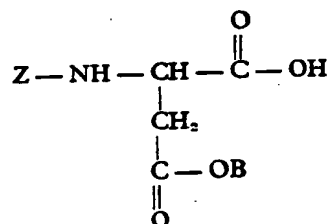
- wherein Z is the amino protecting group trityl, *p*-nitrobenzyloxycarbonyl, *p*-bromobenzyloxycarbonyl, or benzyloxycarbonyl, B is a benzyl radical which forms a protective ester moiety and A is a *p*-nitrophenyl or alkoxy-carbonyl radical forming a labile oxygenated function; with an amino acid ester of the general formula



and followed by removal of the protecting groups from the resulting compound of the formula



21. A process for the preparation of compounds as claimed in claim 1 which comprises contacting a compound of the general formula



with an amino acid ester of formula (C) in the presence of a dehydrating agent, followed by removal of the protecting groups from the resulting compound of formula (D).

22. A process for the preparation of compounds as claimed in claim 1 which comprises contact L-aspartic acid with phosgene and contacting the resulting L - 2,5 - oxazolidinedione - 4 - acetic acid with an amino acid ester of formula (C).

23. A process as claimed in claim 20 or 21 which comprises effecting removal of the protecting groups of formula (D) wherein Z is benzyloxycarbonyl and B is benzyl by catalytic hydrogenolysis.

24. A process as claimed in claim 23 wherein the catalyst is palladium black.

25. A process as claimed in claim 20 for the preparation of L - aspartyl - L - tyrosine methyl ester which comprises contacting N-benzyloxycarbonyl - L - aspartic acid  $\alpha$ -*p*-nitrophenyl,  $\beta$ -benzyl diester with L-tyrosine methyl ester to yield  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl - L - tyrosine methyl ester, which is hydrogenolyzed in aqueous acetic acid, using palladium black catalyst to afford L - aspartyl - L - tyrosine methyl ester.

26. A process as claimed in claim 20 for the preparation of L - aspartyl - L - tyrosine ethyl ester which comprises contacting N-benzyloxycarbonyl - L - aspartic acid  $\alpha$ -*p*-nitrophenyl,  $\beta$ -benzyl diester with L-tyrosine ethyl ester followed by removal of the pro-

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protecting groups by catalytic hydrogenolysis, in aqueous acetic acid, using palladium black catalyst.

- 5 27. A process as claimed in claim 20 for the preparation of L - aspartyl - L - O-methyltyrosine methyl ester which comprises contacting N - benzyloxycarbonyl - L - aspartic acid  $\alpha$ -*p*-nitrophenyl,  $\beta$ -benzyl diester with L-O-methyltyrosine methyl ester hydrochloride followed by removal of the protecting groups by catalytic hydrogenolysis in aqueous acetic acid, using palladium black catalyst.

- 10 28. A process as claimed in claim 20 for the preparation of L - aspartyl - L - O-ethyltyrosine methyl ester which comprises contacting N-benzyloxycarbonyl L-aspartic acid  $\alpha$ -*p*-nitrophenyl,  $\beta$ -benzyl diester with L - O - ethyltyrosine methyl ester followed by removal of the protecting groups by catalytic hydrogenolysis in aqueous acetic acid, using palladium black catalyst.

- 15 29. A process as claimed in claim 20 for the preparation of L - aspartyl - L - tyrosine methyl ester which comprises contacting L-tyrosine methyl ester with the anhydride formed by reacting N - benzyloxycarbonyl-L - aspartic acid  $\beta$ -benzyl ester with ethyl-

chloroformate, followed by removal of the protecting groups by catalytic hydrogenolysis in aqueous acetic acid, using palladium black catalyst. 30

30. A process as claimed in claim 21 wherein dicyclohexylcarbodiimide is used as the dehydrating agent.

31. A process as claimed in claim 21 for the preparation of L - aspartyl - L - tyrosine methyl ester which comprises contacting N-benzyloxycarbonyl - L - aspartic acid  $\beta$ -benzyl ester with L-tyrosine methyl ester in the presence of dicyclohexylcarbodiimide to form  $\beta$ -benzyl N - benzyloxycarbonyl - L - aspartyl-L - tyrosine methyl ester which is then hydrogenolyzed in aqueous acetic acid, using palladium black catalyst to afford L - aspartyl - L-tyrosine methyl ester. 35 40 45

32. A process as claimed in claims 20, 21 and 22 substantially as described with reference to any of the Examples 2 to 10, 12, 14, 16 and 18.

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